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Short communication

Development of analytic microdevices for the detection of phenol using polymer hydrogel particles containing enzyme–QD conjugates

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ABSTRACT

We present the fabrication of a microdevice for the detection of phenol by combining microfluidic channels and poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel microparticles containing tyrosinase–quantum dot conjugates. PHEMA hydrogel microparticles containing conjugates of enzyme (tyrosinase) and quantum dot (QD) were prepared by dispersion photopolymerization and entrapped within a microfilter-incorporated reaction chamber in a microfluidic channel. The fluorescence change, due to the fluorescence quenching effect caused by the enzyme reaction between phenol and tyrosinase, was used to detect phenol. The fluorescence intensity of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates at 585 nm decreased with phenol concentration. In conclusion, the microfluidic channels fabricated in this study entrapping PHEMA hydrogel microparticles containing enzyme–QD conjugates show the potential to be used as an analytic microdevice for the detection of phenol.

1. Introduction

Phenolic compounds are a class of polluting chemicals easily absorbed by animals and humans through the skin and mucous membranes. A considerable number of organic pollutants, widely distributed throughout the environment, have a phenolic structure. Their presence in surface and ground water poses a potential hazard to human health. Therefore, there is a growing interest in selective and sensitive detection of phenolic compounds. However, the conventional techniques for the detection of phenolic compounds, such as chromatographic, fluorimetric, and spectrophotometric methods, are expensive, time-consuming, need skilled operators, and sometimes require preconcentration and extraction steps that increase the risk of sample loss. Thus, much effort has been devoted to the development of simple, sensitive, accurate, and portable devices to determine the environmental presence and concentration of phenolic compounds [1–4]. Among these, biosensors based on tyrosinase, a copper-containing monooxygenase enzyme, are simple and convenient tools for phenol assays due to their high sensitivity, effectiveness, and simplicity [5,6]. However, despite recent efforts to develop tyrosinase-based biosensors for the detection of phenol, few studies have been reported for developing optical biosensors at the micrometer scale. The miniaturization of biosensors has various advantages over conventional analytical devices, including a smaller dead volume and sample consumption, lower cost, greater sensitivity, higher reproducibility and precision, and the potential to create portable diagnostic tools for on-site analysis. In addition, miniaturized devices provide a safe environment in terms of operator handling of toxic and reactive compounds since nano/micro-volumes of sample are sufficient for analysis [7–9].

In this study, we have developed microdevices for the detection of phenol by combining microfluidic channels and poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel microparticles containing tyrosinase–quantum dot conjugates. In general, for biosensors using enzymes, enzymes are immobilized on hard and dry surfaces such as glass, silica, or magnetic surfaces. However, the main problem associated with enzyme immobilization on a hard surface is that the conformational state of many proteins is very labile and unable to withstand the patterning process conditions, eventually losing their native structure and function [10]. In order to solve this problem, we immobilized enzymes within hydrogel microparticles. Immobilization of enzymes within hydrogel microparticles provides a higher density of enzyme than can be achieved via surface immobilization, as well as a protective environment for the enzyme, resulting in maintenance of the stability of the enzyme due to the features of hydrogels, such as hydrophilicity, biocompatibility, permeability and highly cross-linked networks [11–13]. In addition, microparticles are able to be incorporated into microanalytic devices because of their comparable size to that of microchannels [14]. Conjugates of tyrosinase and quantum dot (tyrosinase–QD conjugates) were prepared and encapsulated in PHEMA hydrogel microparticles by a dispersion

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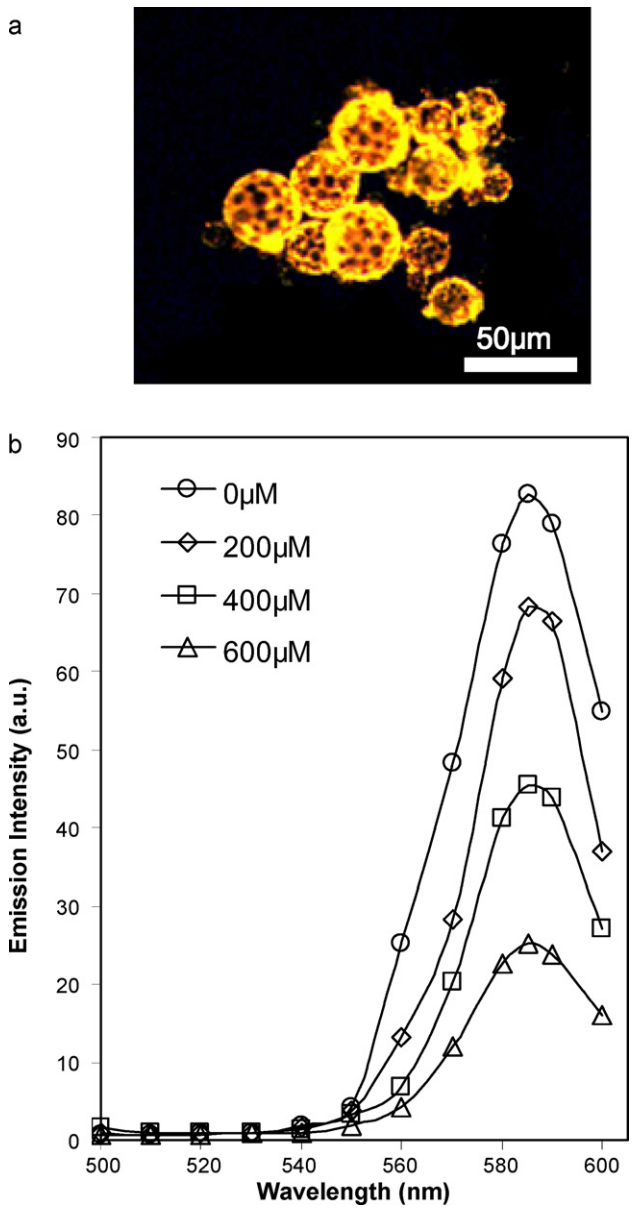

Fig. 1. PHEMA hydrogel microparticles containing tyrosinase–QD conjugates: (a) fluorescence microscope image of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates (excitation at 315 nm) and (b) emission spectra of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates as a function of phenol concentration; phenol concentration (μM) = 0(○), 200(◊), 400(□), and 600(△) (excitation at 315 nm).

photopolymerization. These microparticles were then entrapped within the microfluidic channels fabricated with PDMS in order to make an analytic microdevice. Lastly, the device was tested by investigating the fluorescent change according to the phenol concentration to demonstrate the potential application as an analytic microdevice for the detection of phenol.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA, MW 130), poly(ethylene glycol) dimethacrylate (PEGDMA, MW 550), tyrosinase from mushrooms (5370 units/mg solid), silicon oil, and N-(3-dimethylamioproryl)-N-ethyl carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Carboxyl quantum dots (QD, Qdot® 585 ITK™) were obtained from Invitrogen (Eugene, Oregon, USA). N-hydroxysuccinimide (NHS) and polyethylene glycol trimethylnonyl ether (Tergitol® TMN6) were purchased from Fluka Chemicals (Milwaukee, WI, USA). 1-Hydroxy cyclohexyl phenyl ketone (Irgacure® 184) was obtained from Ciba Specialty Chemicals (Taneatown, NY, USA). Poly(dimethylsiloxane) (PDMS) elastomer was purchased from Dow Corning (Sylgard 184, Midland, MI, USA), which was composed of prepolymer and curing agent.

2.2. Preparation of tyrosinase–QD conjugates

1 mL of tyrosinase solution (2.325 mg/mL in PBS, pH 7.0) and 1 mL of QD solution (0.1 μM in PBS, pH 7.0) were mixed with 1 mL of 20 mM EDC and 1 mL of 40 mM NHS, which were dissolved in PBS. The mixture was then incubated at room temperature for 24 h. During the incubation, the carboxyl groups of QD bonded with lysine residues on the side chain amino groups of tyrosinase through the EDC/sulfo-NHS coupling reaction [15].

2.3. Preparation of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates

PHEMA hydrogel microparticles containing tyrosinase–QD conjugates were synthesized via dispersion photopolymerization of an aqueous monomer mixture in a continuous phase of silicon oil. The formation of PHEMA hydrogel microparticles was based upon the UV initiated free-radical polymerization of methacrylate groups of PHEMA and PEGDMA. Since PEGDMA was used as a cross-linking agent, highly cross-linked PHEMA networks formed. This network represents a three-dimensional structure, capable of entrapping a sensing element and a transducer such as tyrosinase–QD conjugates. The monomer mixture was prepared by mixing 1.4 g of HEMA, 0.072 g (5.0 wt% of monomer) of PEGDMA, 0.036 g (2.5 wt% of monomer) of Irgacure® 184 as an initiator, 0.132 g (9 wt% of monomer) of Tergitol® TMN6 as a dispersion stabilizer, and 1 mL of tyrosinase–QD conjugate solution. The monomer mixture was then added to 20 mL of silicon oil. The mixture of oil and monomer were purged with nitrogen gas for 5 min to remove dissolved oxygen that would act as an inhibitor of the reaction and then stirred using a homogenizer (Ultra Turrax T18 basic, ITK®, Wilmington, NC, USA) at 11,000 rpm for 2 min to form a suspension. For the polymerization, the suspension solution was exposed to 1000 mW/cm^2 of UV light for 300 s. The synthesized particles were separated from the oil by repeated dilution with deionized water and centrifugation at least five times. The washed microparticles were stored in deionized water until future use. The shape and size of the synthesized microparticles were observed using a fluorescence microscope (BX51, Olympus® Co., Japan).

2.4. Fabrication of an analytic microdevice

An analytic microdevice for the detection of phenol was fabricated by combining microfluidic channels and PHEMA hydrogel microparticles containing tyrosinase–QD conjugates. Microfluidic channels in PDMS were obtained by curing a 10:1 mixture of PDMS prepolymer and curing agent against a Si master that had a negative pattern of the desired microchannels defined with SU-8 negative photoresist (MicroChem Co., Newton, MA, USA) [16]. After curing for 5 h at 60 °C, the PDMS replica was removed from the master and oxidized in an oxygen plasma (Harrick Scientific Co., Ossining, NY, USA) with a glass slide for 1 min. Bringing the oxidized PDMS and glass slide into conformal contact resulted in an irreversible seal and thus formed an enclosed microchannel. To make inlet and outlet ports in the microfluidic channel, several holes were punched

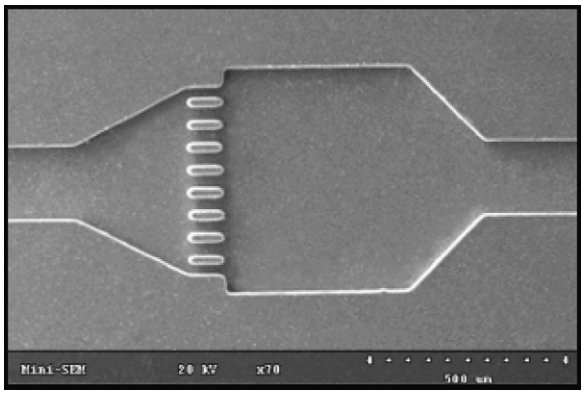

Fig. 2. SEM image of the PDMS device made from a silicon mold. The scale bar is 500 μm.

through the PDMS replica using a 16-gauge needle prior to bonding the PDMS to the glass slide. Polyethylene tubes were inserted into these holes and then connected to a syringe pump (KD Scientific Inc., Holliston, MA, USA). In order to complete the analytic microdevice, synthesized PHEMA hydrogel microparticles containing tyrosinase–QD conjugates were entrapped in the chamber of the microfluidic channel using a syringe pump.

2.5. Fluorescent characterization

The change in fluorescence intensity of QD due to the enzymatic reaction between phenol and tyrosinase was determined using a fluorescence microscope and a fluorescent spectrophotometer (Cary Eclipse, Varian Inc., Palo Alto, CA, USA). QD used in this study have an emission peak at a wavelength of 585 nm. For emission scans, the excitation wavelength was set to 315 nm and the emission wavelengths were scanned from 315 nm to 650 nm. Various concentrations of phenol were introduced to the particles containing tyrosinase–QD conjugates and the microdevice and then fluorescence intensity was measured.

3. Results and discussion

Tyrosinase–QD conjugates were encapsulated in PHEMA hydrogel microparticles by a dispersion photopolymerization. A fluorescence microscope image of the synthesized PHEMA hydrogel microparticles containing tyrosinase–QD conjugates is shown in Fig. 1a. The particles had a spherical shape and emitted a strong orange color when they were excited at 315 nm, which indicated that the tyrosinase–QD conjugates were successfully incorporated within the PHEMA microparticles and the fluorescence property of QD was maintained after the conjugation and encapsulation. In order to verify the preservation of the activities of enzyme and QD during encapsulation and the potential of the particles containing tyrosinase–QD conjugates as an optical biosensor, the emission intensities of the particles were examined by changing the concentration of phenol. Tyrosinase catalyzes the oxidation of phenol and phenol derivatives, in the presence of oxygen, to the respective catechol derivatives that are further oxidized by the enzyme respective quinone intermediates [17,18]. The quinone intermediates act as electron acceptors and reduce the fluorescence intensity of a fluorophore, known as a fluorescence quenching effect. Therefore, in the presence of tyrosinase–QD conjugates and phenol, quinone intermediates produced from the enzymatic reaction between tyrosinase and phenol can reduce the fluorescence intensity of QD [19,20], the emission peak at 585 nm in this study. Finally, it is possible to correlate the enzyme-catalyzed reaction between tyrosinase and phenol with the fluorescent change of the QD. The

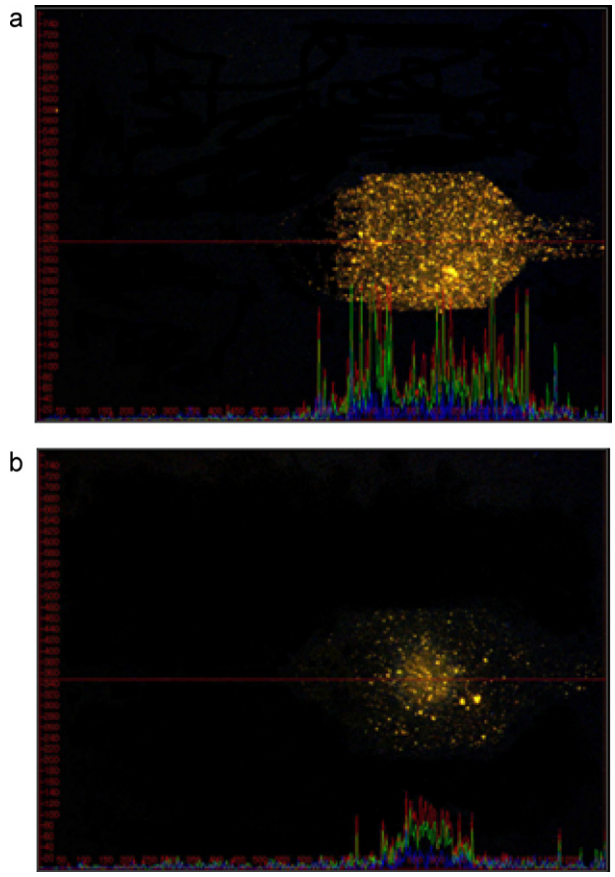

Fig. 3. Fluorescence microscope images of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates entrapped in a reaction chamber in a microfluidic channel: (a) before the phenol solution was introduced and (b) after an 800 μM phenol solution was introduced at a flow rate of 1 μL/min (excitation at 315 nm).

emission spectra of particles containing tyrosinase–QD conjugates as a function of phenol concentration are illustrated in Fig. 1b. The emission peak at 585 nm decreased when the concentration of phenol increased. This is because, as previously mentioned, when the phenol concentration increased, more quinone intermediates were produced by the enzymatic reaction and the emission intensity of QD decreased. Moreover, as a result of monitoring the change in the fluorescent intensity resulting from the enzyme reaction between the tyrosinase in the particles containing tyrosinase–QD conjugates and phenol over time, we found that the fluorescent intensity had not changed significantly over 7 days. These results indicate that tyrosinase–QD conjugates were successfully encapsulated in the PHEMA hydrogel microparticles and they did not lose their activity after they went through polymerization. In addition, in order to investigate the fluorescence recovery of QD in particles containing tyrosinase–QD conjugates, we compared emission intensities of QD in particles containing tyrosinase–QD conjugates when the phenol was added and after removed by washing with PBS. After the phenol was removed, the emission intensity of QD was recovered by 87% compared with the original emission intensity of QD in the particles.

A key feature of the microdevices developed in this study was the microfilter-incorporated reaction chamber. Fig. 2 shows a reaction chamber in the microchannel fabricated with PDMS made from a silicon mold. The in-channel integrated microfilters were incorporated in the reaction chamber to retain and collect PHEMA hydrogel microparticles containing tyrosinase–QD conjugates within the chamber while simultaneously allowing solution to flow through the chamber. The microfilters were composed of rectangular pillar

structures. The distance between pillars was smaller than the average diameter of the particles and thus the microparticles containing tyrosinase–QD conjugates could be trapped within the reaction chamber. In this study, the gap between pillars was designed to be 25 μm, so that the hydrogel microparticles greater than 25 μm were perfectly retained inside the reaction chamber without passing through the microfilter. In addition, since the dimension of the reaction chamber was on a micrometer scale (chamber size 450 μm × 550 μm), collected hydrogel microparticles would be confined to a relatively small area, thereby facilitating detection. To complete the device as an analytic microdevice for detecting phenol, prepared PHEMA hydrogel microparticles containing tyrosinase–QD conjugates were pumped into the reaction chamber with a syringe pump at a flow rate ranging from 2 to 5 μL/min. At this range of flow rate, the microparticles were retained in the chamber without buckling or migrating. Finally, in order to evaluate whether the device fabricated in this study could be used as an analytic microdevice to detect phenol, an 800 μM phenol solution was introduced into the device at a flow rate of 1 μL/min using a syringe pump and the fluorescence response was investigated. Fig. 3 shows fluorescent microscopic images of the reaction chamber filled with PHEMA hydrogel microparticles containing tyrosinase–QD conjugates. As shown by the fluorescence intensity profile on the images, when the phenol solution was introduced, the reaction between tyrosinase and phenol successfully quenched the fluorescence intensity of the QD. These results indicate the potential application of our device as an analytic microdevice for the detection of phenol.

4. Conclusions

For the first step in the development of analytic microdevices for the detection of phenol, PHEMA hydrogel microparticles containing tyrosinase–QD conjugates were prepared via a dispersion photopolymerization and these microparticles were then entrapped within a microfluidic channel fabricated with PDMS. The emission intensity of PHEMA microparticles containing tyrosinase–QD conjugates decreased when the concentration of phenol increased, resulting from the fluorescence quenching effect caused by the enzyme reaction between tyrosinase and phenol, which indicates that the activities of the tyrosinase and QD were maintained when they were conjugated and encapsulated within the PHEMA hydrogel particles. After the microfilter-incorporated reaction chamber was prepared in the microfluidic channels, the particles were entrapped within the chamber and the phenol solution was introduced into the chamber. The fluorescence intensity of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates decreased as phenol was introduced. To conclude, the devices fabricated in this study using polymer hydrogel particles containing enzyme–QD conjugates have the potential to be used as a microdevice for the detection of phenol.

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References

[1] J. Abdullah, M. Ahmad, Y.H. Lee, N. Karuppiah, H. Sidek, Talanta 70 (2006) 527–532.
[2] G. Busca, S. Berardinelli, C. Resini, L. Arrighi, J. Hazard. Mater. 160 (2008) 265–288.
[3] R.S. Freire, N. Duran, L.T. Kubota, J. Braz. Chem. Soc. 13 (2008) 456–462.
[4] S. Girotti, E.N. Ferri, M.G. Fumo, E. Maiolini, Anal. Chim. Acta 608 (2008) 2–29.
[5] B. Kim, Y. Lee, K. Lee, W.G. Koh, Curr. Appl. Phys. 9 (2009) 225–228.
[6] F.N. Ishikawa, B. Stauffer, D.A. Caron, C. Zhou, Biosens. Bioelectron. 24 (2009) 2967–2972.
[7] N. Alexa, H. Zhang, J.R. Lead, Anal. Chim. Acta 655 (2009) 80–85.
[8] S. Gulati, V. Rouilly, X. Niu, J. Chappell, R. Kitney, J.B. Edel, P.S. Freemont, A.J. Demello, J. R. Soc. Interface 6 (2009) 493–506.
[9] M. Mir, A. Homs, J. Samitier, Electrophoresis 30 (2009) 3386–3397.
[10] Y.P. Kim, S.Y. Park, E.K. Oh, Y.H. Oh, H.S. Kim, Biosens. Bioelectron. 24 (2009) 1189–1194.
[11] J.A. Diramio, W.S. Kisaalita, G.F. Majetich, J.M. Shimkus, Biotechnol. Prog. 21 (2005) 1281–1288.
[12] N. Sanabria-DeLong, A.J. Crosby, G.N. Tew, Biomacromolecules 9 (2008) 2784–2791.
[13] B.V. Slaughter, S.S. Khurshid, O.Z. Fisher, A. Khademhosseini, N.A. Peppas, Adv. Mater. 21 (2009) 3307–3329.
[14] Q. Zhang, J.J. Xu, H.Y. Chen, Electrophoresis 27 (2006) 4943–4951.
[15] G.T. Hermanson, Bioconjugate Techniques, second ed., Academic Press, Rockford, 2008.
[16] J.R. Anderson, D.T. Chiu, R.J. Jackman, O. Cherniavskaya, J.C. McDonald, H.K. Wu, S.H. Whitesides, G.M. Whitesides, Anal. Chem. 72 (2000) 3158–3164.
[17] H.B. Yildiz, R. Freeman, R. Gill, I. Willner, Anal. Chem. 80 (2008) 2811–2816.
[18] J. Yuan, W. Guo, E. Wang, Anal. Chem. 80 (2008) 1141–1145.
[19] R. Gill, M. Zayats, I. Willner (Eds.), Angew. Chem. Int. 47 (2008) 7602–7625.
[20] E.I. Sagun, A.A. Yarovoi, A.M. Shulga, V.N. Knyukshto, A.P. Stupak, in: V.E. Borisenko, S.V. Gaponenko, V.S. Gurin (Eds.), Photoinduced Electron Transfer in Heterocomposites Based on Semiconductor Cdse Quantum Dots and Quinines, Physics, Chemistry and Application of Nanostructures, World Scientific Publishing Co., Singapore, 2009, pp. 148–152.